# Macrocyclic Amino Carboxylates as Selective MMP-8 Inhibitors

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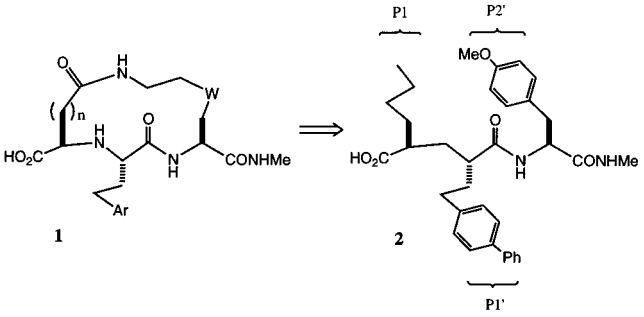
Matrix metalloproteinases (MMPs) are a family of zinc-containing endopeptidases needed for normal turnover and maintenance of the extracellular matrix.[2] Their proteolytic activity is tightly controlled by endogenous tissue inhibitors of metalloproteinases (TIMPs) and the nonspecific α2-macroglobulins.[3] Disruption of this balance and overproduction of the MMPs results in the degradation of the matrix, which includes cartilage and connective tissues. As a result, the MMPs have been implicated in several diseases including arthritis[4] and tumor metastasis.[5] Inhibition of the MMPs, therefore, has gained considerable attention and has become an important strategy in the development of novel therapeutics. However, with the large number of MMPs now characterized, the selective inhibition of the therapeutically relevant MMP(s) will be of paramount importance when considering the potential toxicity of a chronically dosed drug acting on enzymes involved in tissue turnover. We have been interested in, and have recently disclosed, the synthesis of potent carboxylates as broad[6] and selective[7] inhibitors of the MMPs. In an effort to improve potency, selectivity, and metabolic stability of these linear inhibitors, we investigated *macrocyclic amino carboxylates* linked from P1 to P2′, having the general formula **1**. In this communication, we describe the discovery of a novel class of macrocycles as selective MMP-8 inhibitors.

**Scheme 1**




**Design.** In a recent report,[6] we disclosed the X-ray crystal structure of carboxylate **2** cocrystallized with MMP-3.[8] As shown in Figure 1, carboxylate **2** has its P1 (*n*-butyl) and P2′ (*p*-methoxybenzyl) residues extending toward solvent, away from the active site, where they become situated in close proximity to each other. This is a similar observation to that made in the previous paper for succinate hydroxamates.[9] Thus, we surmised the P1 and P2′ groups of an amino carboxylate could be connected to form a macrocycle of varying size. The solvent exposed area is large, and therefore we felt confident that the macrocycle, and various linking groups, would be accommodated there. Amino carboxylates orient themselves differently than the hydroxamates described in the previous paper,[9] and this was taken into account in our modeling. This difference is manifested through the extra atom (nitrogen) in the extended conformation and a distinct mode of ligation to the active site zinc. From modeling, the larger ring amino carboxylates appeared to better preserve the extended conformation and the projection of the carboxylate to the active site zinc. To model this, the 14-membered lactam **12** was docked into the active site of MMP-3. This orientation was refined with 20-ps of molecular dynamics using the procedure of Luty et al.[10] Figure 2 shows the average structure during the final 3-ps of the run, which was overlapped with the crystal structure of carboxylate **2**. This demonstrates that the macrocycle is a good mimic of the overall gross structure and provides for excellent overlap of the carboxylate moieties. Simulations were also carried out on lactam **12** within the active site of MMP-8.[11] The 3-ps average structure from the end of the run is displayed in Figure 3. This representation indicates that the lactam **12** is capable of participating in the typical hydrogen bond network well documented[12] for MMP inhibitors.

**Figure 1.** Carboxylate **2** was cocrystallized in MMP-3.[6] The figure shows the structure of carboxylate **2** extracted from the X-ray crystal structure (carbons are yellow with nitrogen blue and oxygen red).

**Synthesis.** The macrocyclic amino carboxylates were synthesized by the representative route shown in Scheme

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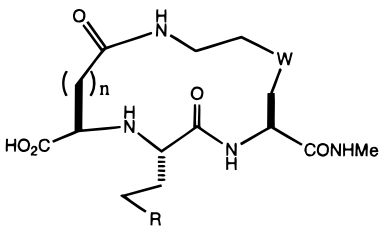
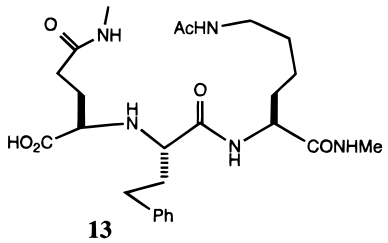
**Table 1.**





**13**

| | | | | $K_i$ (nM)[a] | | | | |
|---|---|---|---|---|---|---|---|---|
| compd | *n* | W | R | MMP-1 | MMP-2 | MMP-3 | MMP-8 | MMP-9 |
| **13** | linear | | | 2860 | 1533 | 14088 | 293 | 404 |
| **11** | 1 | $-CH_2-$ | Ph | 900 | 2400 | 35000 | 531 | 9800 |
| **12** | 2 | $-CH_2-$ | Ph | 2500 | 8100 | 13500 | 17 | 6600 |
| **14** | 2 | $-O_2CCH_2-$ | Ph | 8400 | 238 | 2900 | 10 | 5800 |
| **15** | 2 | $-CH_2-$ | PhPh | 1448 | 249 | 2400 | 9 | 288 |

[a] $K_i$'s were determined as previously described.[9,16]

**Figure 2.** Overlay between the minimized[10] 14-membered lactam **12** (carbons are white with nitrogen blue and oxygen red) and carboxylate **2** from Figure 1 (carbons are yellow with nitrogen blue and oxygen red).

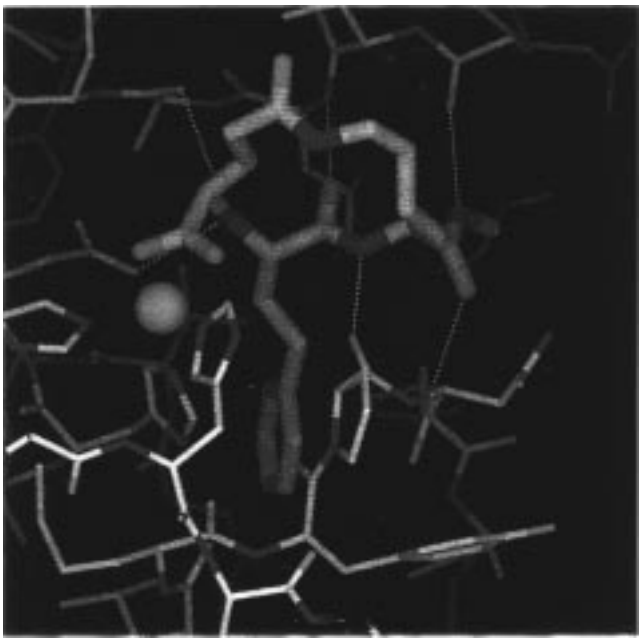


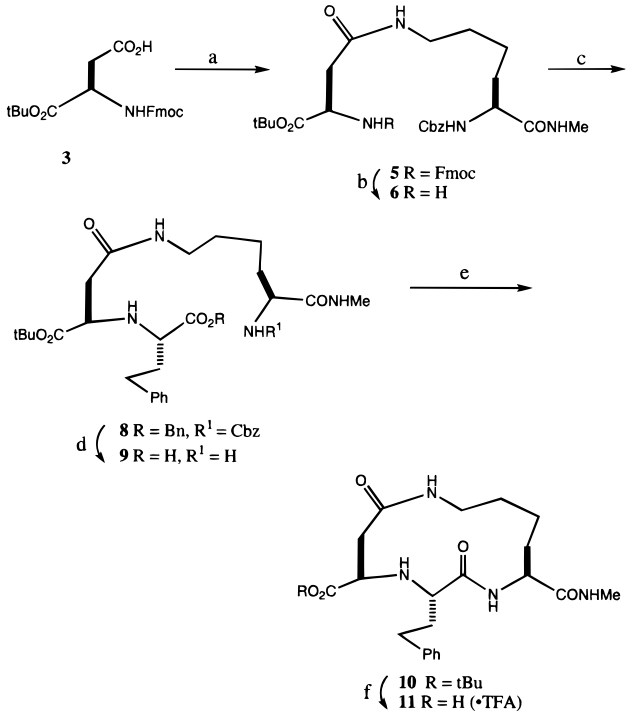
**Figure 3.** The minimized[10] 14-membered lactam **12** (carbons are gray with nitrogen blue and oxygen red) docked into MMP-8 (carbons are white with nitrogen blue and oxygen red; catalytic zinc is orange).

2 for carboxylate **11**. The aspartic acid derivative **3** was coupled to lysine **4** via a standard EDC coupling. The resulting amide **5** was treated with diethylamine to remove the Fmoc group and provide the primary amine **6**. In general, we found that amines of this type could be efficiently alkylated[13] with triflate **7**[14] to afford, in this case, the secondary amine **8**. Catalytic hydrogenation gave the amino acid **9**, which was cyclized under moderate dilution to yield the 13-membered lactam **10**. All that remained to complete the synthesis was removal of the *tert*-butyl group via TFA, thus providing the target macrocyclic amino carboxylate **11**.

**Results and Discussion.** The smallest macrocycle synthesized was the 13-membered lactam **11**, which displayed less affinity for MMP-2, -3, -8, and -9 when compared to the model, linear compound **13** (Table 1). As discussed above, the larger rings appeared superior from modeling, and therefore we synthesized the 14-membered lactam **12**. A 30-fold increase in activity toward MMP-8 was observed for **12** when compared to the 13-membered lactam **11**. In addition, the selectivity of **12** for MMP-8 was enhanced as compared to the other MMPs we screened. In keeping with this trend, we synthesized the 16-membered lactam **14**. Again, an increase in activity toward MMP-8 was observed for **14** versus the 14-membered lactam **12**. However, **14** was more potent against MMP-2, -3, and -9, and therefore less attractive than the selective **12**.

Another way to gain affinity in this class of inhibitors is to install biaryl groups in P1′.[6,15] The resulting biaryl lactam **15** was only slightly more active toward MMP-8 than the parent **12**. The main limitation of the biaryl, however, is the enhanced affinity toward other MMPs with large S1′ pockets. In fact, this was observed for **15**, as the inhibition of MMP-2, -3, and -9 increased dramatically. It is interesting to note that the biaryl had only a modest effect on MMP-1, known to have a restricted S1′.

**Scheme 2**[a]




[a] Reagents: (a) EDC, HOBt, NMM, L-Cbz-Lys-NHMe **4**, $CH_2Cl_2$ (47%); (b) $Et_2NH$, DMF (86%); (c) (*R*)-Ph$(CH_2)_2$(TfO)$CHCO_2$Bn **7**, *i*-$Pr_2NEt$, $CH_2Cl_2$, (78%); (d) $H_2$, Pd/C, MeOH; (e) BOP, *i*-$Pr_2NEt$, DMF (33%, two steps); (f) TFA, $CH_2Cl_2$ (95%).

**Conclusion.** In this communication, we demonstrated that linear, amino carboxylate MMP inhibitors can be transformed into macrocyclic inhibitors linked from P1 to P2′. We have discovered that ring size has an effect on activity, and that the larger rings were potent MMP-8 inhibitors. With the proper ring size and substituents, potent and selective MMP-8 inhibitors have been described. These unique structures should lead to a better understanding of ligand binding in the MMPs, as well as aid in the development of future inhibitors.

**Supporting Information Available:** Experimental details and spectroscopic data are available for the synthesis of compounds **5**−**11** (4 pages). Ordering information is given on any current masthead page.

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